These findings make several important contributions. First, they provide additional data showing the critical regulatory roles that miRNAs play in cancer biology (Ventura and Jacks, 2009) and, in particular, stem cell biology. Recently, in human embryonic stem cells, increased expression of miR-145 in clusters of these cells called embryoid bodies was shown to target the 3′UTRs of mRNAs encoding pluripotency factors, resulting in the promotion of differentiation and loss of self-renewal (Xu et al., 2009). In the new study, identical miRNA clusters are suppressed in both normal mammary epithelial stem cells and human breast tumor-initiating cells, demonstrating the close similarities in molecular regulatory controls that are used by stem cells and their malignant counterparts. Again, it is shown that stem cell regulatory pathways are the same as cancer stem cell regulatory pathways. Second, the new data demonstrate an important additional layer of regulatory control that is exerted on BMI1. As the miRNA clusters are located on three distinct chromosomal regions, the authors rightly point out the advantages of such an organization, providing layers of back up to restrain BMI1 activity in inappropriate cell populations.

As with many other miRNA studies and because this is a new and rapidly evolving field, many questions remain. Obviously, many other targets of these three miRNA clusters exist and the importance of these other targets in determining the effects of expression of the miRNA clusters remains to be defined. Mechanisms that regulate the expression of miRNAs during stem cell differentiation are not known. Also, functional data are shown only for one of the miRNA species, and it would be valuable to know the effectiveness of different members of the miR-200c cluster in suppressing *BMI1* translation or effecting *BMI1* mRNA degradation. Although the effects of miR-200c also are shown in a teratocarcinoma (Tera-2) cell line, it is likely that completely distinct milieus exist for miRNA expression and targeting in distinct cell types, and it is not clear whether targeting BMI1 is as important in these cell types. As well, the promotion of differentiation by miR-200c in Tera-2 cells may be a unique effect in this particular cell type. A role for miR-200c in the induction of differentiation in normal mammary epithelial stem cells is less clear, and it is not known whether miR-200c is actually required for the production of differentiated cell types or just blocks self-renewal.

The Shimono et al. study is the first to identify roles for specific miRNAs in parallel populations of stem cells and in their neoplastic counterparts. This field of research is likely to be a rich but complex arena for studying mechanisms of stem cell function, particularly in cancer stem cells. It will be especially interesting to determine whether miRNA genes are directly targeted, either alone or cooperatively, during cancer initiation in normal stem cells or progenitor cells, or whether they are altered later in the neoplastic process and so contribute to tumor progression. The new work also suggests that therapeutic approaches involving manipulation of miRNA expression may be another strategy to target cancer stem cells.

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Alpha Cells Beget Beta Cells

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DOI 10.1016/j.cell.2009.07.022

Understanding the origins of insulin-producing beta cells of the pancreas could lead to new treatments for diabetes. Collombat et al. (2009) now show that in response to injury, a population of pancreatic progenitor cells can give rise to glucagon-expressing alpha cells that then transdifferentiate into beta cells.

Diabetes results from loss of the insulin-producing beta cells of the pancreatic islets. A clear identification of the progenitor cell population that gives rise to beta cells and an understanding of the factors and the cellular mechanisms that govern beta cell regeneration may lead to new and more effective treatments for diabetes. The paired homeobox transcription factor Pax4 has been implicated in the control of endocrine cell fate during pancreas development. Mice lacking Pax4 have diminished numbers of beta cells and instead accumulate alpha cells, a cell type that expresses the hormone

glucagon (Sosa-Pineda et al., 1997). In this issue, Collombat et al. (2009) demonstrate that the alpha cell lineage is endowed with an unusual plasticity after tissue injury: when Pax4 is ectopically expressed in the alpha cells of mice at a dose equivalent to its physiological level, it instructs these cells to become functional beta cells (Figure 1).

The major function of alpha cells is to produce the hormone glucagon, which, in counter-regulation to the actions of insulin, stimulates hepatic glucose production to maintain blood glucose levels during fasting (Gromada et al., 2007). In prior work, it has been shown that glucagon-expressing cells are the earliest identifiable cells of the endocrine lineage during pancreas development, suggesting that glucagon-expressing cells may be endocrine progenitor cells (Gromada et al., 2007). The study by Collombat et al. now provides unequivocal evidence for progenitor cells that exist in the tissue surrounding the duct linings of pancreas. These progenitor cells express the transcription factor neurogenin 3 (Ngn3), proliferate, and adopt an alpha cell identity. They then rapidly transdifferentiate into insulin-secreting beta cells. The rapid transition from alpha cell to beta cell results in a relative deficiency of alpha cells and a subsequent loss of glucagon signaling. The loss of glucagon signaling stimulates the expression of Ngn3 in the progenitor cells associated with the duct and promotes their amplification. The increase in Ngn3 expression in progenitor cells appears to be a response to the increased expression of Pax4 in alpha cells and to the rapid depletion of alpha cells by their transition to beta cells. Evidence for this comes from the administration of exogenous glucagon to the Pax4 transgenic mice, which suppresses the proliferation of Ngn3-positive cells and their subsequent transition to alpha and then beta cells. Conversely, mice lacking the glucagon receptor (Vuguin et al., 2006) display enhanced production of alpha cells. By using interfering RNAs to specifically inhibit Ngn3 activation, Collombat et al. show that the formation of new beta cells in response to the ectopic expression of Pax4 occurs via a molecular pathway that involves the activation of Ngn3, similar to the pathway that operates during embryonic development of the pancreas. A similar approach was used by Xu et al. (2008) in their seminal discovery of the critical role of Ngn3 in the specification of endocrine progenitor cells in the adult pancreas in response to injury.

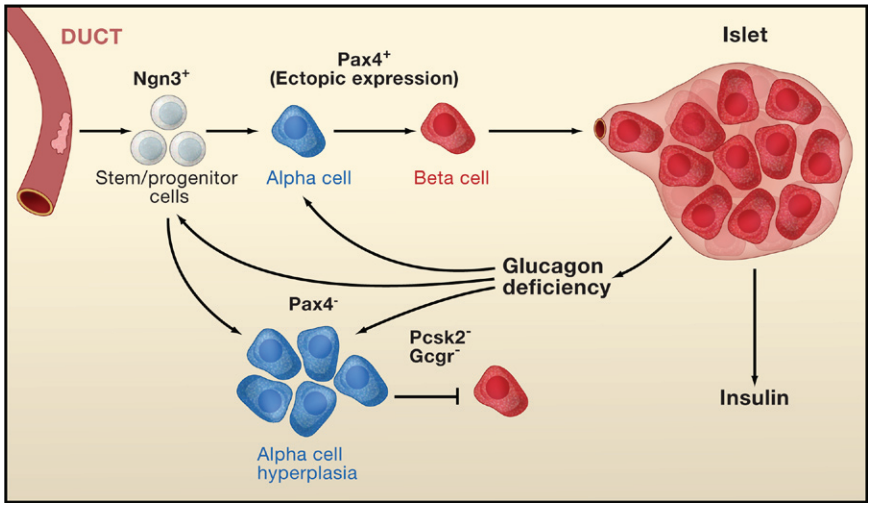

Figure 1. The Formation of New Insulin-Producing Beta Cells in the Adult Pancreas
Endocrine progenitor cells expressing the transcription factor neurogenin 3 (Ngn3) arise from the duct lining of the pancreas. These cells differentiate into glucagon-expressing alpha cells and, in the presence of ectopic transgenic expression of the transcription factor Pax4, rapidly transdifferentiate into insulin-producing beta cells. This rapid transformation of alpha cells into beta cells results in a deficiency of alpha cells and of glucagon signaling, which further stimulates the formation and expansion of Ngn3-positive stem cells and thereby increases the formation of new beta cells. Notably, other mouse models characterized by deficient glucagon signaling display marked alpha cell hyperplasia. These models include mice lacking the glucagon receptor (Gcgr) or the prohormone convertase 2 (Pcsk2). However, in the absence of ectopic expression of Pax4, alpha cells do not efficiently transdifferentiate into beta cells.

During embryogenesis, the *glucagon* gene (Gcg) is expressed in the earliest endocrine cells that can be detected. The findings of Collombat et al. challenge the previously held notion that mature insulin-producing cells do not derive from cells that originally express glucagon (Herrera, 2000). Collambat et al. use lineage-tracing experiments to assess islet regeneration in transgenic mice after administration of the toxin streptozotocin, which destroys beta cells. They show that the vast majority of newly formed beta cells originate from cells that previously expressed *glucagon.* This finding provides a convincing demonstration that alpha cells retain the potential to convert to beta cells in the adult pancreas. Moreover, the latent stem/progenitor cells adjacent to the ducts respond to alpha cell depletion by activating the formation and expansion of facultative endocrine progenitors. A decrease in the number of Ngn3-labeled cells and loss of beta cell hyperplasia occurs in *Pax4*-misexpressing mice treated with glucagon, underscoring the importance of glucagon in the feedback inhibition of alpha cell neogenesis.

Interestingly, adaptive hyperplasia of endocrine cells often occurs by the sustained actions of trophic factors or a diminution of factors that suppress feedback inhibition. In pathophysiological conditions, such as sustained high glucose due to the development of insulin resistance in diabetes, beta cells exhibit hyperplasia in an attempt to produce more insulin to counteract hyperglycemia. Likewise, sustained hypoglycemia and hypoglucagonemia results in marked alpha cell hyperplasia. Of note, disruption of glucagon signaling, such as knockout of the glucagon receptor (Vuguin et al., 2006) or deficiency of Pax4 (Sosa-Pineda et al., 1997), results in alpha cell hyperplasia. Paradoxically, alpha cell hyperplasia also occurs in conditions of insulin deficiency that result from injury of beta cells. For example, this occurs in mice (Li et al., 2000) or monkeys (Dufrane et al., 2009) given streptozotocin, or in NOD mice in which beta cells are injured by an autoimmune attack (Ogawa et al., 2004). In all of these models, the alpha cells appear to be blocked from, or incapable of, transformation into beta cells. The observations

of Collombat et al. demonstrate that Pax4 alone is sufficient to overcome this block and raise the possibility that deficiency of glucagon or glucagon signaling may serve as a cue for the regeneration of both the alpha cells and duct-associated progenitor cells.

In the transgenic mouse model studied by Collombat et al., the dramatic expansion of the beta cell mass throughout postnatal life is attributed to a continuous formation of beta cells through alpha cell reprogramming rather than the slow self-replication of pre-existing mature beta cells (Dor et al., 2004). These observations provide proof that the adult pancreas has latent capabilities to regenerate new beta cells in response to injury and do so by resurrecting developmental programs to allow amplification of progenitor cells and selective lineage commitments. In this case, the beta cells originating from cells that express glucagon are fully functional and counter diabetes induced by streptozocin. This study will redirect thinking in the field of regenerative medicine in the treatment of diabetes, which has been focused on the premise that beta cell neogenesis only occurs through the replication of existing beta cells (Dor et al., 2004).

Finally, these studies show that replacement of endocrine tissue by transplantation of insulin-producing cells, derived from embryonic stem cells or other cells, is not the only feasible approach to a permanent treatment for diabetes. To the contrary, it is now possible to contemplate treatment approaches that coax pre-existing, latent stem/progenitor cells and alpha cells to make new beta cells.

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Data Harvesting from Fields of Spindles

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DOI 10.1016/j.cell.2009.07.019

The mitotic spindle is essential for chromosome segregation and must be large enough to accommodate all of the chromatin in the dividing cell. In this issue, Dinarina et al. (2009) grow "fields" of spindles on coverslips to investigate the relationship between chromatin and spindle size as well as intrinsic mechanisms of spindle assembly.

The main function of the mitotic spindle is to accurately segregate replicated chromosomes during cell division. Spindle size varies little between cells of the same type but does not always scale with cellular dimensions. This point is strikingly illustrated by the amphibian egg during meiosis when the diameter of the egg is roughly 30 times longer than the length of the spindle. Thus, the steady-state dimensions of the spindle are not always constrained by the geometry of the cell and must instead be determined by intrinsic mechanisms. In this issue of *Cell*, Dinarina et al. (2009) use an innovative approach involving growing "fields" of spindles in *Xenopus* extracts to examine the role of chromatin in modulating spindle assembly and size.

How is spindle size set? In order to ensure genomic fidelity during cell division, a spindle must be large enough to attach to, align, and segregate all of the chromosomes within the cell. This suggests that spindle size should scale with the amount of chromatin or at least with the area that it occupies on the metaphase plate. One way to achieve this proportionality is for chromatin to dictate where and to what extent the microtubules that make up the spindle will polymerize. Indeed, it is now known that spindle microtubules form locally in response to at least two chromatin-based signaling pathways (Kalab et al., 1999; Sampath et al., 2004).

The determination of spindle size is also a mechanical problem that requires consideration of the forces generated by the dynamics of microtubule polymerization/depolymerization and by the motors that move along microtubules using them